IMMUNOCHEMICAL DETECTION OF PROTEINS IN THE SMALL SUBUNIT OF RAT LIVER RIBOSOMES INVOLVED IN BINDING OF THE TERNARY INITIATION COMPLEX

U.-A. BOMMER, F. NOLL, G. LUTSCH* and H. BIELKA
Central Institute of Molecular Biology, GDR Academy of Sciences, Department of Cell Physiology and
**Department of Electron Microscopy, 1115 Berlin-Buch, GDR*

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1. Introduction

An essential step of peptide chain initiation in eukaryotes is the binding of the ternary initiation complex [Met-tRNA$_f$ × eIF-2 × GTP] to the P site of small ribosomal subunit, thus forming the quaternary initiation complex [40 S subunit × Met-tRNA$_f$ × eIF-2 × GTP] [1–4].

First attempts to analyze proteins of the small subunit of rat liver ribosomes involved in binding of components of the ternary initiation complex have been made by the application of antibodies [5] and by crosslinking experiments [6]. In [5] we described the effect of antibodies against ribosomal protein S3 (according to the newly proposed common nomenclature of eukaryotic ribosomal proteins [7]) on the binding of [^{3}H]Met-tRNA$_f$ in complex with eIF-2 and GTP to the 40 S ribosomal subunit. We now report further experiments with antibodies against 9 proteins of the small ribosomal subunit. From the strong inhibitory activity of the antibodies against ribosomal proteins S3, S6, and S13 and their location on the small ribosomal subunit as studied by immune electron microscopy ([8,9] and unpublished) it is concluded that these proteins are involved in the P site organization and that the P site is located in the head region of the small ribosomal subunit.

2. Materials and methods

Pure single proteins of the small subunit of rat liver ribosomes were prepared as in [10]. Antibodies against these proteins were raised in rabbits [10] and chicken [9] and purified by immune affinity chromatography ([9] and unpublished). No immunological crossreactions could be detected between any of the proteins used [10].

Small ribosomal subunits, [^{3}H]Met-tRNA$_f$ (15 000 dpm/pmol) and eukaryotic initiation factor 2 (eIF-2) were prepared from rat liver as in [11]. For these studies, eIF-2 was further purified by an additional gel filtration step (Sephadex G-200).

Ternary initiation complexes [[^{3}H]Met-tRNA$_f$ × eIF-2 × GMPPCP] were formed in 100 μl samples containing 80 mM KCl, 2 mM β-mercaptoethanol, 25 mM Tris (pH 7.5), 0.16 mM GMPPCP, ~10 pmol [^{3}H]Met-tRNA$_f$ and 20 μg eIF-2 by incubation for 7 min at 37°C.

Subsequently, the ternary complexes were mixed with 40 S ribosomal subunits or 40 S–antibody complexes, respectively; $MgCl_2$ was added to 3.5 mM final conc. and the incubation continued for 4 min at 37°C to allow quaternary initiation complex formation. These complexes were then analyzed by sucrose gradient centrifugation (10–80% sucrose in solution of 80 mM KCl, 5 mM $MgCl_2$, 2 mM β-mercaptoethanol, 20 mM Tris (pH 7.5); Spinco rotor SW 60, 60 000 rev./min for 80 min at 2°C) (detailed in [11]). The amount of Met-tRNA$_f$ bound to 40 S ribosomal subunits was calculated from the specific activity of the [^{3}H]Met-tRNA$_f$ sample and the radioactivity in the 40 S subunit fraction in the gradients.

In the experiments with antibodies against ribosomal proteins (fig.2) samples of 14 μg 40 S ribosomal subunits were preincubated in a buffer of 100 mM KCl, 1.5 mM $MgCl_2$ and 20 mM Tris (pH 7.5) with different amounts of antibodies for 30 min at 0°C. The volume of the samples varied from 50–100 μl

depending on the antibody concentrations. The 40 S ribosomal subunit–antibody complexes were then mixed with simultaneously preformed ternary initiation complexes and assayed for quaternary complex formation as above.

3. Results and discussion

The formation of the quaternary initiation complex [40 S subunit × Met-tRNA$_f$ × eIF-2 × GTP] is usually measured by binding of radioactivity labeled Met-tRNA$_f$ to 40 S ribosomal subunits in the presence of eIF-2 and GTP (or GMPPCP). That eIF-2 is really bound to the small ribosomal subunit and thus involved in the assembly of the quaternary initiation complex has been shown by the factor dependence of Met-tRNA$_f$ binding [3,4,11], by experiments with labeled eIF-2 in the reticulocyte system [12,13], and by chemical crosslinking [6]. From results of experiments with labeled and unlabeled eIF-2, demonstrated in fig.1, it furthermore becomes evident that eIF-2 along with Met-tRNA$_f$ is bound to the small ribosomal subunit. Therefore, it can be concluded that binding of labeled Met-tRNA$_f$ also reflects eIF-2 binding.

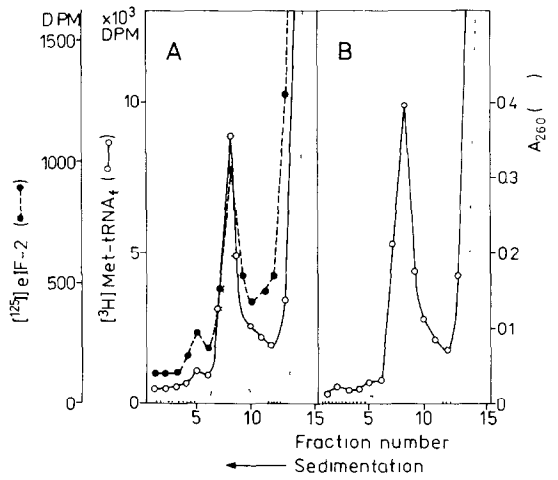

Fig.1. Binding of ^{125}I-labeled eIF-2 to 40 S subunits. The formation of the quaternary initiation complexes [40 S × eIF-2 × GMPPCP × Met-tRNA$_f$] was analysed on sucrose gradients as in section 2. The incubation mixture contained: (A) 36 μg [^{125}I] eIF-2 (2000 dpm/μg protein); (B) 39 μg unlabeled eIF-2. [^{3}H]Met-tRNA$_f$, (10 pmol) and 28 μg 40 S subunits were present in (A,B). Labeling of eIF-2 with ^{125}I according to [14] was performed in our department by Dr P. Westermann.

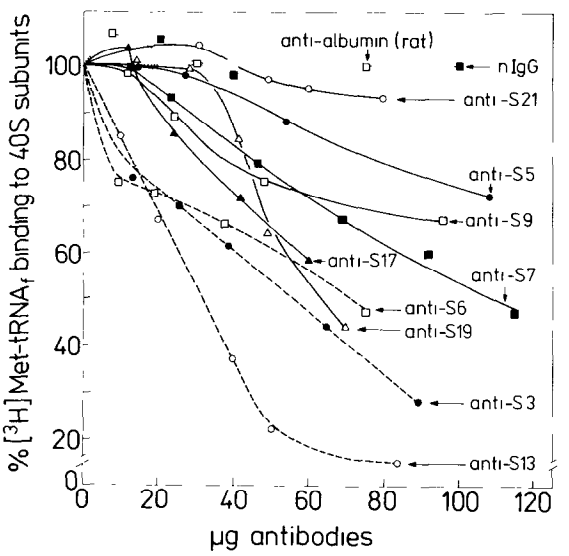

Fig.2. Inhibition of [^{3}H]Met-tRNA$_f$ binding to small ribosomal subunits by antibodies against pure ribosomal proteins. eIF-2-dependent binding of [^{3}H]Met-tRNA$_f$ to 40 S subunits in the presence of the indicated amounts of antibodies was assayed as in section 2. Each point demonstrates [^{3}H]Met-tRNA$_f$ binding to the 40 S subunit in relation to the control experiment without antibodies. 100% corresponds to [^{3}H]Met-tRNA$_f$ binding to 14 μg 40 S subunits over 1.5–2.0 pmol (nIgG, immunoglobulin G fraction from non-immune antiserum).

Fig.2 shows the inhibition of binding of the ternary initiation complex containing [^{3}H]Met-tRNA$_f$ to the small ribosomal subunit by antibodies against various pure ribosomal proteins. As to the efficiency of the blocking activity 3 groups of antibodies can be roughly distinguished:

(i) Antibodies against ribosomal proteins S13, S3 and S6 inhibit ternary complex binding very strongly;

(ii) Antibodies against proteins S5, S7, S9, S17 and S19 exert weaker inhibition effects;

(iii) Antibodies against ribosomal protein S21 as well as against rat serum albumin and the nIgG fraction (controls) do not show any effect, not even at higher concentrations.

The possibility that the inhibition effects are caused by precipitation can be excluded because the interaction of antibodies used here with the 40 S ribosomal subunits has no effect on their migration in sucrose gradients (as shown, e.g., for anti-S3 antibody in fig.2 of [5]).

Because of the very strong blocking effect of antibodies against proteins S3, S6 and S13, we suppose that at least these proteins are involved in interactions with components of the ternary initiation complex and thus should contribute to the formation of the P-site on the small subunit of eukaryotic ribosomes. This assumption is supported by the fact that eIF-2 can be crosslinked, amongst others, to proteins S3 and S13 [6].

For the proteins whose antibodies exhibit a very strong inhibition effect it can be suggested that domains of the polypeptide chains of these proteins are involved directly in binding of the ternary initiation complex. The weaker inhibitory effect of the antibodies of the second group may be due to the location of the corresponding proteins either only partially at this region or in its neighbourhood. At least in the latter case the inhibition might be caused by an unspecific hindrance of the complex binding by the relatively large IgG molecule.

The behaviour of some antibodies, e.g., anti-S19, which are active at higher concentrations only, might be due to the presence of different antibodies within the given antibody population characterized by differences in their affinities to the corresponding antigenic determinant of the ribosomal protein. The biphasic inhibition curve could be explained by the assumption that at lower concentrations at first the antibodies of higher affinity are bound, which do not interfere with eIF-2 binding. At higher concentrations, however, also the antibodies with lower affinity react and cause inhibition of the binding of the ternary initiation complex, because the corresponding antigenic determinant is located at or near the P-site. Therefore, it can be assumed that besides proteins S3, S6, and S13 also S19 is involved, at least with parts of its polypeptide chain, in the organization of the P-site of the 40 S ribosomal subunit.

In immune electron microscopic studies we showed ([8,9] and unpublished) that proteins S3 and S6 have closely neighboured antigenic determinants in the head region of the small ribosomal subunit. These findings, together with the results obtained for other proteins, are demonstrated in fig.3. Neighbourhoods of proteins determined by immune electron microscopy could be confirmed by crosslinking experiments. Proteins S2 and S3 as well as S3 and S3a were found as crosslinked pairs (P. Westermann et al. in preparation). Proteins S3 and S3a can be crosslinked to eIF-2 [6]. However, antibody binding sites of protein

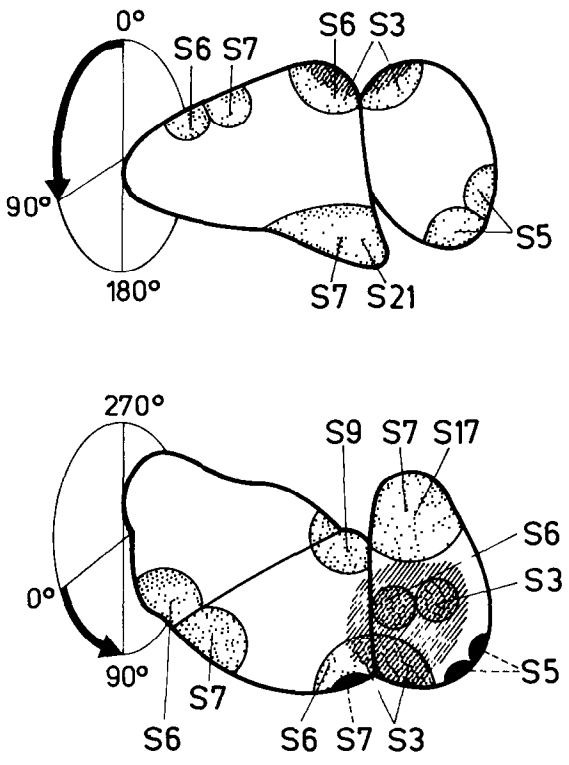

Fig.3. Presumptive localization of the eIF-2 binding site on the 40 S ribosomal subunit (///////) and the position of the antigenic determinants of ribosomal proteins ((⊛) upper level; (•) lower level of projection) as determined by immune electron microscopic studies ([8,9] and unpublished).

S21 are located in a region distant from the antigenic determinants of proteins S3 and S6. This observation is in agreement with the finding that antibodies against S21 do not inhibit binding of the ternary initiation complex to the small ribosomal subunit (see fig.2).

Therefore, in summary, it seems justified to conclude that the P-site of the small ribosomal subunit is located mainly in its head region (fig.3), at least as far as proteins S3 and S6 are concerned. Besides these two proteins, proteins S13 and S19 seem to be involved in binding of the ternary initiation complex.

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